ligand on T cells. Moreover, cytokines, IL-2 and IL-4 also enhance CD40 ligand expression on anti-CD3 stimulated T cells, indicating that there is a cooperative regulation between costimulatory molecules and cytokines in mediating immune responses in vivo. Furthermore, the CD40-CD40 ligand interaction increases Th cell-dependent antibody responses, proinflammatory cytokine production, and is required for macrophage tumoricidal and microbicidal activities. In particular, CD40 ligand is not expressed on resting T cells, but induced by CD3-TCR triggering processes. CD40 (45- to 50- kDa glycoprotein) is a member of the TNF receptor superfamily and is expressed on B cells, monocytes, and dendritic cells. However, its ligand, CD40 ligand (gp39) is a type II transmembrane protein with sequence homology to TNF-α and transiently expressed on activated T cells. However, interaction of adhesion molecule, LFA-3 on T cells with ICAM-1 on APC is highly regulated by conformation change of LFA-3 that with a high affinity and avidity for ICAM-1. It has been known that costimulation of LFA-3 in the context with CD3 monoclonal antibody or Class II plus antigen results in T cell proliferation and higher production of a variety of cytokines from T cells. Furthermore, interaction of LFA-3 with ICAM-1 triggers signal pathways. As compared to costimulatory CD40/CD40 ligand molecules, co-localization of these adhesion molecules and anti-CD3 or anti-TCR antibody on the surface is necessary for proper signaling to T cells.

Coinjecting APC-stimulating or attracting molecules along with DNA vaccines results in a more efficient induction of both arms of immunity. In intramuscular (i.m.) injection, DNA is taken up into myofibers with subsequent endogenous expression, leading to presentation of a natural form of antigen to the immune system. Secreted antigens are ingested by phagocytosis and then presented as a peptide-MHC II complex by macrophages which can provide the primary activation signal, costimulatory ligands and cytokines necessary for stimulation of naive T cells. Recent evidence also supports direct transfection of APC in vivo following either i.m. or skin delivery of DNA vaccines. Coinjection of DNA vaccines with costimulatory molecules, such as B7.1 and B7.2, dramatically enhanced antigen-specific cellular immune responses, such as Th cell proliferation responses and cytotoxic T cell activities. Similarly, coinjection with GM-CSF genes enhances both antibody and cellular immune responses in the viral DNA

vaccine models. Coinjection with pLacZ plus CD40 ligand cDNAs enhances both humoral and cellular, in particular CTL in an antigen-dependent manner. However, there have been no HSV challenge studies via codelivery with costimulatory and adhesion molecules. It would be also interesting to compare these two different pathways in induction of antigen-specific immune responses and protective immunity against HSV-2.

We observed that no significant increase in gD-specific IgG production through vaccine modulation with the CD40 and CD40 ligand genes. However, this is not compatible with previous finding that coinjection with CD40 ligand enhanced antibody production to an antigen when delivered with DNA vector (β-galactosidase). This discrepancy might be due to the nature of the antigens tested. However, there is a similar finding in which the similar IgG isotype production pattern was induced by coinjection with CD40 ligand coinjection. In our studies, codelivery with CD40 ligand induced a significant increase in IgG2a production, as compared to IgG1 isotype which is believed to be mediated by Th1 type immune responses. This implies that polarization of gD-specific immune responses to Th1 type is achieved by coinjecting plasmid vectors driving expression of CD40 ligand. In contrast, significant increase in gD-specific IgG production was observed by coinjection with LFA-3, as compared to gD DNA vaccine alone or ICAM-1 coinjection. This indicates that LFA-3 could enhance antibody responses in vivo. We also observed that LFA-3 coinjection enhanced production of both Th1 and Th2 isotypes indicated by increased production of IgG1 and IgG2a isotypes, implying that LFA-3 could drive both Th1 and Th2 immune responses in vivo.

Increased Th cell proliferation was achieved by coinjecting plasmid DNAs encoding CD40 ligand. This is again compatible with previous findings in other models that CD40 ligand molecules increase antigen-specific Th cell proliferation and IFN-γ production as well as CTL responses . This pattern is in line with the cytokine production levels we observed as coinjection with CD40 ligand cDNA enhanced both IL-2 and IFN-γ secretion, but inhibited IL-10 production. Thus, the use of the CD40 ligand cDNA in gD DNA vaccination was effective for polarizing the immune responses towards a Th1 phenotype, increasing cell-mediated immunity. However, coinjection with LFA-3 enhanced production of IL-2, IL-10 and IFN-γ while coinjection with ICAM-1

slightly enhanced the cytokine production. This supports the IgG isotype pattern that LFA-3 drives immune responses to both Th1 and Th2 phenotypes.

Chemokines have been recently reported to play an important role in a manner reminiscent of cytokines in the immune and inflammatory responses. Ocular inflammatory disease mediated by HSV infection was suppressed by topical administration of Th2 type cytokine protein (IL-10). This application resulted in suppressed chemokine production. The disease (inflammation in the eye) was also ameliorated by injection with anti-MIP-1α but not MCP-1, indicating that MIP-1α again segregates as a Th1 type chemokine. However, the role of chemokines on infectious status is under investigation. In this study, production of RANTES and MIP-1α was enhanced by codelivery of CD40 ligand higher than CD40, suggesting that CD40 ligand molecules play an important role in regulating β chemokine production from T cells. Both LFA-3 and ICAM-1 enhanced production of MIP-1α and RANTES. In contrast, MCP-1 production was unaffected by LFA-3 or inhibited by ICAM-1, indicating that adhesion molecules could also regulate β chemokine production in vivo.

It has been reported that humoral, cellular or both immune responses could be responsible for protective immunity against HSV infection. Passive immunization with HSV-specific monoclonal antibodies resulted in protection from lethal HSV infection. During viral infection, neutralizing antibodies can inactivate free viral particles, but are not able to inhibit intracellular HSV infection. It appears that antibody-dependent, complement-mediated and antibody-dependent cell-mediated cytotoxicity (ADCC) are insufficient to control HSV infection . Thus, it has been suggested that HSV-specific cellular-mediated immunity may play a major effector function to eradicate HSV-infected cells and control HSV infection. Importance of cellular immune responses mediated by $CD4^+$ and/or $CD8^+$ T cells on control of HSV infection has been well documented

We observed that coinjection with CD40 ligand molecules induces significantly enhanced protection from mortality resulting from HSV-2 infection. This suggests that there is a positive correlation between protective immunity and Th1 type cellular immunity, which is supported by increased Th cell proliferation responses and IFN-γ production levels when coinjected with CD40 ligand molecules. Our observation is

compatible with the previous finding that coinjection with CD40 ligand enhances protective immunity against challenge with *Leishmania major* or with metastatic tumor expressing antigen. We also observed that LFA-3 induces significantly enhanced protection from mortality resulting from HSV-2 infection. It seems that LFA-3 enhancement of cellular and/or humoral immunity is responsible for reducing HSV-2-derived mortality in this system. It is also possible that CD40 ligand or LFA-3-induced IFN-γ might be partially responsible for the anti-HSV-2 activity in vivo. Thus, CD40 ligand and LFA-3-driven cellular or humoral mediated immunity appears to be correlated with protection from HSV infection.

In conclusion, the data presented here suggest that costimulatory and adhesion molecules have different costimulatory pathways in the induction of antigen-specific immune responses. In particular, CD40 ligand drives immune responses to a Th1 type while LFA-3 favors both Th1 and Th2 immune types. Such activities have previously only been associated with cytokines. These data indicate that costimulatory molecules have as central role as cytokines in the induction of antigen-specific immunity. Also CD40 ligand and LFA-3 mediate enhanced protection against lethal HSV-2 challenge in gD DNA vaccination. This finding broadens our weapons for infectious diseases.

Example 7

We analyzed modulatory effects of chemokines (IL-8, IP-10, RANTES, MCP-1, MIP-1α) on immune phenotype and protection against lethal challenge with HSV-2. We observed that IL-8 and RANTES coinjection dramatically enhanced antigen-specific immune responses and protection from lethal HSV-2 challenge. However, coinjection with MCP-1 and IP-10 increased mortality of the challenged mice. These studies demonstrate that chemokines can dominate and drive immune responses in a manner more reminiscent of cytokines, playing an important role in the generation of protective antigen-specific immunity.

The initiation of immune or inflammatory reactions is a complex process involving the coordinated expression of costimulatory molecules, adhesion molecules, cytokines, and chemokines. In particular, chemokines are important in the molecular regulation of trafficking of immune cells to the peripheral sites of host defenses. The

chemokine superfamily consists of two subfamilies based upon the presence (α family) or absence (β family) of a single amino acid sequence separating two cysteine residues . α and β chemokines have been shown to induce direct migration of various immune cell types, including neutrophils, eosinophils, basophils, and monocytes. Recently, the (chemokine family (CXC type), interleukin (IL)-8 and interferon-γ inducible protein (IP)-10, and the β chemokine family (CC type), RANTES (regulated on activation, normal T cell expressed and secreted), monocyte chemotactic protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α have been shown to chemoattract T lymphocytes. In particular, IL-8 and IP-10 have been known to chemoattract neutrophils, inducing them to leave the bloodstream and migrate into the surrounding tissues. Similarly, RANTES chemoattracts monocytes, unstimulated $CD4^+/CD45RO^+$ memory T cells and stimulated $CD4^+$ and $CD8^+$ T cells. MIP-1α has been known to chemoattract and degranulate eosinophils . MIP-1α also induces histamine release from basophils and mast cells and chemoattacts basophils and B cells . MCP-1 is an important chemokine in chronic inflammatory disease. MCP-1 induces monocytes to migrate from the bloodstream to become tissue macrophages. MCP-1 also chemoattracts T lymphocytes of the activated memory subset. Recent studies support that chemokine receptors mark T cell subsets and that chemokines may be involved in the generation of antigen-specific immune responses .

To investigate the modulation of immune responses and protective immunity, we co-delivered a DNA expression construct encoding HSV-2 gD protein with plasmids encoding for chemokines (IL-8, IP-10, RANTES, MCP-1, MIP-1α). We then analyzed their modulatory effects in antigen-specific immune induction and protection from challenge. We first investigated the *in vivo* effects of selected chemokines on the induction of antigen-specific antibody responses. As controls we immunized animals with gD vaccine and 2 proinflammatory cytokines, TNF family genes (TNF-α and TNF-β). These proinflammatory cytokines were studied as they are thought to similarly be involved in early immune responses and should serve as positive controls. ELISA was used to measure levels of systemic gD-specific IgG in mice (Balb/c) immunized with DNA vectors. Each group of mice (n=10) was immunized with gD DNA vaccines (60 μg per mouse) plus chemokine genes (40 μg per mouse) or TNF genes (40 μg per

mouse) at 0 and 2 weeks. DNA constructs expressing gD antigens and chemokines were previously cloned (Pachuk, et al. 1998 Current topics Microbiol. Immunol. 226, 79; Kim, et al. 1998 J. Clin. Invest. 102, 1112; and Kim, et al. 1998 Eur. J. Immunol. 28, 1089). The mice were bled 2 weeks after the second immunization, and then equally pooled sera per group were serially diluted for reaction with gD. The ELISA titers were determined as the reverse of the highest sera dilution showing the same optical density as sera of naive mice. The absorbance (O.D.) was measured at 405 nm. ELISA titers of equally pooled sera collected 2 weeks post the second immunization were determined as 12,800 for IL-8, 6,400 for IP-10, 6,400 for RANTES, 6,400 for MCP-1, 12,800 for MIP-1α, 25,600 for TNF-α, 6,400 for TNF-β and 6,400 for the gD DNA vaccine alone. This shows that coinjection with IL-8 and MIP-1α genes results in a moderate, but not significant enhancement of gD-specific IgG antibodies. In contrast, IP-10, RANTES or MCP-1 showed similar levels of antibody responses to that of pgD vaccination alone. The TNF-α cDNA control resulted in systemic IgG levels significantly higher than those of gD DNA vaccine alone.

It has been reported that induction of the IgG1 isotype is induced by Th2 type cytokines, whereas the IgG2a isotype production is influenced and driven in vivo by Th1 type cytokines . This has been used as an indicator for determining if immune responses are under control of Th1 or Th2 cytokines. We analyzed the IgG subclasses induced by the coinjections. IgG isotypes induced by each immunization group were measured. Each group of mice (n=10) was immunized with gD DNA vaccines (60 μg per mouse) plus chemokine genes (40 μg per mouse) or TNF genes (40 μg per mouse) at 0 and 2 weeks. The mice were bled 2 weeks after the last immunization and then sera were diluted to 1:100 for reaction with gD. For the determination of relative levels of gD-specific IgG subclasses, anti-murine IgG1, IgG2a, IgG2b, or IgG3 conjugated with HRP (Zymed, San Francisco, CA) were substituted for anti-murine IgG-HRP. The absorbance (O.D.) was measured at 405 nm. The relative optical density was calculated as optical density of each IgG subclass/total optical density. Line bars represent the mean (n=10) of relative optical densities of each mouse IgG subclass. The relative ratios of IgG2a to IgG1 (Th1 to Th2) were measured. The pgD immunized group had a IgG2a to IgG1 ratio of 0.62. Coinjection with IL-8, RANTES or TNF-α genes increased the

relative ratio of gD-specific IgG2a to IgG1 to 0.8. On the other hand, coinjection with IP-10 and MIP-1α decreased the relative ratio of IgG2a to IgG1 (0.3 and 0.4), whereas co-immunization with MCP-1 or TNF-β genes resulted in an IgG subtype pattern similar to pgD vaccination alone. This analysis supports that IL-8 and RANTES drive immune responses towards Th1 phenotype in vivo in a similar manner to γ-IFN type cytokines. Thus, these results extend prior findings in the HIV model that the shift in humoral immune responses to either Th1 or Th2 could be modulated by chemokines, again suggesting that chemokines can modulate cytokine production in vivo.

Th cell proliferation is a standard parameter used to evaluate the potency of cell-mediated immunity. We measured Th cell proliferative responses following co-immunization with chemokine genes by stimulating splenocytes from immunized animals *in vitro* with gD proteins. Th-cell proliferation levels of splenocytes after *in vitro* gD stimulation in mice (Balb/c) coimmunized with α-chemokine cDNA , β-chemokine cDNA and the TNF controls. Each group of mice (n=2) was immunized with gD DNA vaccines (60 μg per mouse) plus chemokine genes (40 μg per mouse) or TNF genes (40 μg per mouse) at 0 and 2 weeks. Two weeks after the last DNA injection, two mice were sacrificed and spleen cells were pooled for the proliferation assay. Splenocytes were stimulated with 1 and 5 μg of gD-2 proteins per ml and 5 μg of PHA per ml as a positive control. After 3 days of stimulation, the cells were harvested and the cpm was counted. Samples were assayed in triplicate. The PHA control sample showed a stimulation index of 40-50. pgD DNA vaccination alone resulted in gD-specific Th cell proliferative responses. We also observed the significant enhancement of Th cell proliferative responses over that of gD DNA vaccine alone by co-injection with IL-8, RANTES and TNF-α cDNAs. We also observed a slight enhancement in proliferation by coinjection with TNF-β genes. In contrast, co-immunization with IP-10, MCP-1 and MIP-1α genes appeared to have minimal effects on the levels of Th cell proliferative responses. However, the coinjections showed no effects on PHA-induced non-specific Th cell proliferative responses (S.I. range was 40 to 50). The gD plasmid vaccination does not result in CTL responses due to a lack of CTL epitope in the Balb/c background. However, to evaluate cellular effects in more detail we next examine cytokine production profiles.

Th1 cytokines (IL-2 and IFN-γ) and Th2 cytokines (IL-4, IL-5 and IL-10) have been a mainstay in our understanding of the polarization of immune responses. Th1 immune responses are thought to drive induction of cellular immunity, whereas Th2 immune responses preferentially drive humoral immunity. Based on the IgG phenotype results we further evaluated the Th1 vs Th2 issue by analyzing directly cytokine release. As shown in Table 4, IL-2 production was dramatically increased almost 7 fold by coinjection with IL-8 cDNA. IL-2 was also induced by coinjection with TNF-α cDNA, and by coinjection with the MIP-1α cassette. In particular, production of IFN-γ was most significantly enhanced by codelivery of RANTES, 20 fold and IL-8, 6 fold, further supporting the isotyping results and demonstrating that IL-8 and RANTES mediate Th1 type cellular immune responses in an antigen-dependent fashion. RANTES, IL-8, TNF-α, and TNF-β coinjections also enhanced IL-10 production significantly higher than pgD vaccine alone. This illustrates that IL-8 and RANTES drive T cells of predominantly Th1 over a Th2 type.

To determine if chemokine coinjection could induce β chemokine production in an antigen-dependent manner, we coimmunized and then analyzed release levels of β chemokines of splenocytes after in vitro stimulation with recombinant gD antigen or control antigen. As shown in Table 5, MCP-1 production was dramatically increased by coinjection with IL-8 cDNA, but was decreased by coinjection with RANTES and MIP-1α cassettes. In particular, production of MIP-1α is most significantly enhanced by codelivery of RANTES and IL-8. In the case of RANTES, IL-8 and RANTES coinjections enhanced RANTES production higher than pgD vaccine alone. This indicates that RANTES modulates antigen-specific immune responses differently from IL-8 in the HSV model. This also supports that chemokines modulate their own production.

HSV is the causative agent of a spectrum of human diseases, such as cold sores, ocular infections, encephalitis, and genital infections. HSV can establish viral latency with frequent recurrences in the host. During viral infection, neutralizing antibody inactivates viral particles, but is unable to control intracellular HSV infection. Rather, cellular-mediated immunity plays a major effector function for eradication of HSV-infected cells and spread of HSV in vivo. Adoptive transfer of cytotoxic T

lymphocyte (CTL) raised against HSV results in complete protection from lethal HSV challenge in animals. Furthermore, there have been several reports that Th1 type $CD4^+$ T cells play more crucial role for protection from HSV-2 challenge. When $CD4^+$ T cells were depleted in vivo, protective immunity against HSV was lost . Moreover, Th1 type $CD4^+$ T cells generate a large amount of IFN-γ . IFN-γ upregulates class I and II expression on HSV-infected cells to allow better recognition by cytotoxic $CD4^+$ T cells and CD8+ CTL, and has direct anti-HSV effects. Codelivery with Th1 type cytokine cDNAs enhanced survival from lethal HSV-2 challenge while codelivery with Th2 type cytokine cDNAs worsened the disease status. Similarly, protection enhanced by codelivering with a prototypic Th1 type cytokine IL-12 cDNA was mediated Th1 type $CD4^+$ T cells in HSV challenge model, underscoring the importance of Th1 type T cell-mediated protective immunity against HSV infection.

It is important that antigen-specific immune modulation influences pathogen replication. Survival rates of mice (Balb/c) immunized with gD DNA vaccines plus α chemokine cDNA, β chemokine cDNA and the TNF controls were measured. Each group of mice (n=8) was immunized with gD DNA vaccines (60 μg per mouse) plus chemokine genes (40 μg per mouse) or TNF genes (40 μg per mouse) at 0 and 2 weeks. Three weeks after the second immunization, the mice were challenged i.vag. with 200 LD_{50} of HSV-2 strain 186 (7×10^5 PFU). Before inoculating the virus, the intravaginal area was swabbed with a cotton tipped applicator (Hardwood Products Company, Guiford, ME) soaked with 0.1 M NaOH solution and then cleaned with dried cotton applicators. Mice were then examined daily to evaluate survival rates. Surviving mice were counted for 61 days following viral challenge. This was repeated once with the expected results We analyzed protective efficacy of chemokine coinjection in the murine herpes challenge model. Mice were coimmunized i.m. with DNA vectors at 0 and 2 weeks and then challenged with HSV-2 at 3 weeks post second immunization. Intravaginal challenge route was chosen as HSV-2 infects mucocutaneously. Immunization with gD DNA vaccine alone resulted in 63% of survival of mice from intravaginal challenge with 200 LD_{50} of HSV-2. Coinjection with IL-8 and RANTES cDNA increased the survival rate to 88%, an almost 30% enhancement of protection rate, whereas coinjection with MCP-1 and IP-10 decreased the survival rate to 25%, more

than a 50% reduction in overall survival from the gD vaccine alone. Similarly, MIP-1α coinjection also negatively influenced the survival rate of vaccinated animals. These observations are striking if one considers the total number of animals tested in each chemokine group (survival rates of gD alone, 11 of 18, 61%; survival rates of IL-8, 17 of 18, 94%; survival rates of IP-10, 5 of 18, 28%; survival rates of RANTES, 17 of 18 94%; survival rates of MCP-1, 6 of 18, 33%; survival rates of MIP-1α, 8 of 18, 44%). This indicates that coinjection with IL-8 and RANTES chemokine gene enhances protection from lethal HSV challenge while coinjection with IP-10 and MCP-1 and to a less degree MIP-1α make animals more susceptible to viral infection in spite of the induction of immune responses. This supports that chemokines IL-8 and RANTES enhanced protection from HSV-2 infection through antigen-specific immune modulation. These studies support that chemokines can act and modulate important immune responses and disease progression in a manner reminiscent of cytokines (Th1 vs Th2). Significant immune modulation could be achieved through the use of codelivered chemokine cDNAs, impacting not just an immune responses but also disease protection. Furthermore, use of chemokine gene-delivered adjuvants, in particular IL-8 and RANTES could be important in crafting more efficacious vaccines or in immune therapies for HSV. We previously reported that coinjection with Th1 type cytokine gene enhances protection rate from lethal HSV challenge while Th2 type cytokine coinjection increases susceptibility of animal to viral infection. In pathogenesis studies, the importance of Th1-like cytokine response for resistance from pathogenic infection has been reported. Thus, it seems likely that Th1 and/or Th2 type immune responses are being driven by these chemokines, resulting in an impact on protection from HSV infectious challenge based on the quality of the immune responses.

We compared the protective efficacy of TNF family coinjection in the herpes challenge model. Coinjection with both TNF-α and TNF-β genes also reduced the rate of survival of challenged mice to 25%, more than 50% reduction in overall survival from the gD vaccine alone. Although gD-specific antibody and Th cell proliferation levels as well as cytokine production levels (IL-2, IFN-γ, IL-10) of mice co-injected with TNF-α genes were much higher than those of gD DNA vaccination alone, TNF cytokine-mediated susceptibility to HSV-2 infection was observed in those animals. The

reason for this observation is unclear but strongly supports that the quality of the responses is significantly important for controlling pathogenic infection.

In conclusion, the data presented here demonstrate that chemokines could modulate immune responses to Th1 and/or Th2 types in an antigen-dependent fashion. Such activities have been previously only been associated with cytokines, implying that chemokines have as central a role as cytokines in the induction of antigen-specific immunity. The use of chemokines to modulate immune responses for immune therapies and vaccination is worthy of further investigation.

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Journal: J. Biol. Chem. 270 (9), 4312-4317 (1995)

ICE
Accession: U13699
Author: Alnemri, E.S., et al.
Journal: J. Biol. Chem. 270 (9), 4312-4317 (1995)

VLA-1
Accession: X17033
Author: Takada., et al.
Journal: J. Biol. Chem. 109(1), 397-407 (1989)

CD86 (B7.2)
Accession: U04343
Author: Azuma, et al.
Journal: Nature. 366 (6450),76 (1993)

Table 2

Picornavirus Family

Genera: Rhinoviruses: (Medical) responsible for ~ 50% cases of the common cold.
Etheroviruses: (Medical) includes polioviruses, coxsackieviruses, echoviruses, and human enteroviruses such as hepatitis A virus.
Apthoviruses: (Veterinary) these are the foot and mouth disease viruses.

Target antigens: VP1, VP2, VP3, VP4, VPG

Calcivirus Family

Genera: Norwalk Group of Viruses: (Medical) these viruses are an important causative agent of epidemic gastroenteritis.

Togavirus Family

Genera: Alphaviruses: (Medical and Veterinary) examples include Senilis viruses, RossRiver virus and Eastern & Western Equine encephalitis.
Reovirus: (Medical) Rubella virus.

Flariviridue Family

Examples include: (Medical) dengue, yellow fever, Japanese encephalitis, St. Louis encephalitis and tick borne encephalitis viruses.

Hepatitis C Virus: (Medical) these viruses are not placed in a family yet but are believed to be either a togavirus or a flavivirus. Most similarity is with togavirus family.

Coronavirus Family: (Medical and Veterinary)
Infectious bronchitis virus (poultry)
Porcine transmissible gastroenteric virus (pig)
Porcine hemagglutinating encephalomyelitis virus (pig)
Feline infectious peritonitis virus (cats)
Feline enteric coronavirus (cat)
Canine coronavirus (dog)
The human respiratory coronaviruses cause ~40 cases of common cold. EX. 224E, 0C43
Note - coronaviruses may cause non-A, B or C hepatitis

Target antigens:
E1 - also called M or matrix protein
E2 - also called S or Spike protein
E3 - also called HE or hemagglutin-elterose glycoprotein (not present in all coronaviruses)
N - nucleocapsid

Rhabdovirus Family
Genera: Vesiliovirus
Lyssavirus: (medical and veterinary) rabies
Target antigen: G protein
N protein

Filoviridue Family: (Medical)
Hemorrhagic fever viruses such as Marburg and Ebola virus

Paramyxovirus Family:
Genera: Paramyxovirus: (Medical and Veterinary)
Mumps virus, New Castle disease virus (important pathogen in chickens)
Morbillivirus: (Medical and Veterinary)
Measles, canine distemper
Pneuminvirus: (Medical and Veterinary)
Respiratory syncytial virus

Orthomyxovirus Family (Medical)
The Influenza virus

Bungavirus Family
Genera: Bungavirus: (Medical) California encephalitis, LA Crosse
Phlebovirus: (Medical) Rift Valley Fever
Hantavirus: Puremala is a hemahagin fever virus
Nairvirus (Veterinary) Nairobi sheep disease
Also many unassigned bungaviruses

Arenavirus Family (Medical)
LCM, Lassa fever virus

Reovirus Family
Genera: Reovirus: a possible human pathogen
Rotavirus: acute gastroenteritis in children
Orbiviruses: (Medical and Veterinary)
Colorado Tick fever, Lebombo (humans) equine encephalosis, blue tongue

Retrovirus Family
Sub-Family:
Oncorivirinal: (Veterinary) (Medical) feline leukemia virus, HTLVI and HTLVII
Lentivirinal: (Medical and Veterinary) HIV, feline immunodeficiency virus, equine infections, anemia virus
Spumavirinal

Papovavirus Family
Sub-Family:
Polyomaviruses: (Medical) BKU and JCU viruses
Sub-Family:
Papillomavirus: (Medical) many viral types associated with cancers or malignant progression of papilloma
Adenovirus (Medical)
EX AD7, ARD., O.B. - cause respiratory disease - some adenoviruses such as 275 cause enteritis

Parvovirus Family (Veterinary)
Feline parvovirus: causes feline enteritis
Feline panleucopeniavirus
Canine parvovirus
Porcine parvovirus

Herpesvirus Family
Sub-Family: alphaherpesviridue
Genera: Simplexvirus (Medical)
HSVI, HSVII
Varicellovirus: (Medical - Veterinary) pseudorabies - varicella zoster
Sub-Family - betaherpesviridue
Genera: Cytomegalovirus (Medical)
HCMV
Muromegalovirus
Sub-Family: Gammaherpesviridue
Genera: Lymphocryptovirus (Medical)
EBV - (Burkitts lympho)
Rhadinovirus
Poxvirus Family
Sub-Family: Chordopoxviridue (Medical - Veterinary)
Genera: Variola (Smallpox)
Vaccinia (Cowpox)
Parapoxivirus - Veterinary
Auipoxvirus - Veterinary
Capripoxvirus
Leporipoxvirus
Suipoxvirus
Sub-Family: Entemopoxviridue

Hepadnavirus Family
Hepatitis B virus
Unclassified
Hepatitis delta virus

Table 3

Bacterial pathogens

Pathogenic gram-positive cocci include: pneumococcal; staphylococcal; and streptococcal. Pathogenic gram-negative cocci include: meningococcal; and gonococcal.

Pathogenic enteric gram-negative bacilli include: enterobacteriaceae; pseudomonas, acinetobacteria and eikenella; melioidosis; salmonella; shigellosis; hemophilus; chancroid; brucellosis; tularemia; yersinia (pasteurella); streptobacillus moniliformis and spirillum; listeria monocytogenes; erysipelothrix rhusiopathiae; diphtheria; cholera; anthrax; donovanosis (granuloma inguinale); and bartonellosis.

Pathogenic anaerobic bacteria include: tetanus; botulism; other clostridia; tuberculosis; leprosy; and other mycobacteria. Pathogenic spirochetal diseases include: syphilis; treponematoses: yaws, pinta and endemic syphilis; and leptospirosis.

Other infections caused by higher pathogen bacteria and pathogenic fungi include: actinomycosis; nocardiosis; cryptococcosis, blastomycosis, histoplasmosis and coccidioidomycosis; candidiasis, aspergillosis, and mucormycosis; sporotrichosis; paracoccidiodomycosis, petriellidiosis, torulopsosis, mycetoma and chromomycosis; and dermatophytosis.

Rickettsial infections include rickettsial and rickettsioses.

Examples of mycoplasma and chlamydial infections include: mycoplasma pneumoniae; lymphogranuloma venereum; psittacosis; and perinatal chlamydial infections.

Pathogenic eukaryotes

Pathogenic protozoans and helminths and infections thereby include: amebiasis; malaria; leishmaniasis; trypanosomiasis; toxoplasmosis; pneumocystis carinii; babesiosis; giardiasis; trichinosis; filariasis; schistosomiasis; nematodes; trematodes or flukes; and cestode (tapeworm) infections.

Table 4. Production levels of IL-2, IL-10 and IFN-γ of splenocytes after in vitro gD stimulation[a]

Immunization group	IL-2 (pg/ml)	IFN-γ (pg/ml)	IL-10 (pg/ml)
Naive	16.7 ± .0.8	10.5 ± 0.7	17.1 ± 6.12
pgD+ pCDNA3	134.7 ± 3.5	22.4 ± 2.4	57.1 ± 4.4
pgD+ IL-8	756.4 ± 5.4	138.5 ± 4.7	128 ± 13
pgD+ IP-10	143.5 ± 3.9	31.5 ± 2.5	69.9 ± 1.9
pgD+ RANTES	59.9 ± 1.1	520 ± 13	360 ± 46.5
pgD+ MCP-1	93.6 ± 4.7	17.9 ± 0.5	49.7 ± 2.3
pgD+ MIP-1α	345.4 ± 18	55.4 ± 1.8	22 ± 2.1
pgD+ TNF-α	403 ± 13.3	77 ± 6.3	86.8 ± 6.2
pgD+ TNF-β	288 ± 5.6	20.8 ± 1.5	78.3 ± 3.6

[a]Each group of Balb/c mice (n=2) was immunized with gD DNA vaccines (60 μg per mouse) plus chemokine genes (40 μg per mouse) or TNF cDNAs (40 μg per mouse) at 0 and 2 weeks. Two weeks after the last DNA injection, two mice were sacrificed and spleen cells were pooled. A 1 ml aliquot containing 6 x 10^6 splenocytes was added to wells of 24 well plates. Then, 1 μg of HSV-2 gD protein/ml was added to each well. After 2 days incubation at 37°C in 5% CO_2, cell supernatants were secured and then used for detecting levels of IL-2, IL-10, and IFN-γ using commercial cytokine kits (Biosource, Intl., Camarillo, Ca.) by adding the extracellular fluids to the cytokine-specific ELISA plates. Samples were assayed in triplicate and the values represent means of released cytokine concentrations ± standard deviation. This represents one of three separate experiments showing the expected result.

Table 5. Production levels of MCP-1, MIP-1α, and RANTES of splenocytes after in vitro gD stimulation[a]

Immunization group	MCP-1 (pg/ml)	MIP-1α (pg/ml)	RANTES (pg/ml)
Naive	153.8 ± 5.7	247 ± 11	769 ± 7
pgD+ pCDNA3	234 ± 5.3	747 ± 39	817 ± 55
pgD+ IL-8	322 ± 24	1,411 ± 113	1,284 ± 53
pgD+ IP-10	246.3 ± 2.7	1,407 ± 459	831 ± 52
pgD+ RANTES	189.7 ± 0	2,267 ± 219	1,077 ± 32
pgD+ MCP-1	209.2 ± 6.4	725 ± 501	646 ± 45
pgD+ MIP-1α	142.7 ± 3.3	787 ± 94	690 ± 39

[a]Each group of Balb/c mice (n=2) was immunized with gD DNA vaccines (60 μg per mouse) plus chemokine genes (40 μg per mouse) at 0 and 2 weeks. Two weeks after the last DNA injection, two mice were sacrificed and spleen cells were pooled. A 1 ml aliquot containing 6 x 10^6 splenocytes was added to wells of 24 well plates. Then, 1 μg of HSV-2 gD protein/ml was added to each well. After 2 days incubation at 37°C in 5% CO_2, cell supernatants were secured and then used for detecting levels of RANTES, MCP-1 and MIP-1α using commercial chemokine kits (R&D Systems, Minneapolis, Md.) by adding the extracellular fluids to the cytokine or chemokine-specific ELISA plates. Samples were assayed in triplicate and the values represent means of released chemokine concentrations ± standard deviation. This represents one of three separate experiments showing the expected result.

Claims

1. A plasmid comprising a nucleotide sequence that encodes an immunogen operably linked to regulatory elements and a nucleotide sequence that encodes an immunomodulating protein operably linked to regulatory elements, wherein said immunomodulating protein is selected from the group consisting of: MCP-1, MIP-1α, MIP-1β, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, and Caspase ICE.

2. The plasmid of claim 1 wherein said immunogen is a target protein that encodes a pathogen antigen, a cancer-associated antigen or an antigen linked to cells associated with autoimmune diseases.

3. The plasmid of claim 1 wherein said immunogen is a pathogen antigen.

4. The plasmid of claim 1 wherein said immunogen is an HIV-1 antigen.

5. The plasmid of claim 1 wherein said immunomodulating protein is ICAM-1 and further comprising a nucleotide sequence that encodes CD86 protein operably linked to regulatory elements.

6. An injectable pharmaceutical composition comprising the plasmid of claim 1.

7. A method of inducing an immune response in an individual against an immunogen comprising administering to said individual a plasmid of claim 1.

8. A plasmid comprising a nucleotide sequence that encodes a herpes simplex antigen operably linked to regulatory elements and a nucleotide sequence that encodes an

immunomodulating protein operably linked to regulatory elements, wherein said immunomodulating protein is selected from the group consisting of: IL-8, RANTES, LFA-3, and CD40L.

9. The plasmid of claim 1 wherein said herpes simplex antigen is HSV2gD.

10. An injectable pharmaceutical composition comprising the plasmid of claim 8.

11. A method of immunizing an individual against a herpes simplex virus infection comprising administering to said individual a plasmid of claim 8.

12. A composition comprising two plasmids:

a first plasmid comprising a nucleotide sequence that encodes an immunogen operably linked to regulatory elements; and

a second plasmid comprising a nucleotide sequence that encodes an immunomodulating protein operably linked to regulatory elements, wherein said immunomodulating protein is selected from the group consisting of: MCP-1, MIP-1α, MIP-1β, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, and Caspase ICE.

13. The composition of claim 12 wherein said immunogen is a target protein that encodes a pathogen antigen, a cancer-associated antigen or an antigen linked to cells associated with autoimmune diseases.

14. The composition of claim 12 wherein said immunogen is a pathogen antigen.

15. The composition of claim 12 wherein said immunogen is an HIV-1 antigen.

16. The composition of claim 12 wherein said immunomodulating protein is ICAM-1 and further comprising a nucleotide sequence that encodes CD86 protein operably linked to regulatory elements, wherein said first plasmid, said second plasmid or a third plasmid comprises said nucleotide sequence that encodes CD86 protein.

17. An injectable pharmaceutical composition comprising the composition of claim 12.

18. A method of inducing an immune response in an individual against an immunogen comprising administering to said individual a composition of claim 12.

19. A composition comprising two plasmids:

a first plasmid comprising a nucleotide sequence that encodes a herpes simplex antigen operably linked to regulatory elements; and

a second plasmid comprising a nucleotide sequence that encodes IL-8, RANTES, LFA-3 or CD40L.

20. The composition of claim 19 wherein said herpes simplex antigen is HSV2gD.

21. An injectable pharmaceutical composition comprising the composition of claim 19.

22. A method of immunizing an individual against a herpes simplex virus infection comprising administering to said individual a plasmid of claim 19.

23. A recombinant vaccine comprising a nucleotide sequence that encodes an immunogen operably linked to regulatory elements and a nucleotide sequence that encodes an immunomodulating protein operably linked to regulatory elements, wherein said immunomodulating protein is selected from the group consisting of: MCP-1, MIP-1α, MIP-1β, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3,

CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, and Caspase ICE.

24. The recombinant vaccine of claim 23 wherein said immunogen is a target protein that encodes a pathogen antigen, a cancer-associated antigen or an antigen linked to cells associated with autoimmune diseases.

25. The recombinant vaccine of claim 23 wherein said immunogen is a pathogen antigen.

26. The recombinant vaccine of claim 23 wherein said immunomodulating protein is ICAM-1 and further comprising a nucleotide sequence that encodes CD86 protein operably linked to regulatory elements.

27. A method of inducing an immune response in an individual against an immunogen comprising administering to said individual a recombinant vaccine of claim 1.

28. The recombinant vaccine of claim 23 wherein said recombinant vaccine is a recombinant vaccinia vaccine.

29. The recombinant vaccine of claim 23 wherein said immunogen is a pathogen antigen.

30. A method of inducing an immune response in an individual against an immunogen comprising administering to said individual a recombinant vaccine of claim 23.

31. A live attenuated pathogen comprising a nucleotide sequence that encodes immunomodulating protein operably linked to regulatory elements, wherein said immunomodulating protein is selected from the group consisting of: MCP-1, MIP-1α,

MIP-1β, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, and Caspase ICE.

32. A method of immunizing an individual against a pathogen comprising administering to said individual the live attenuated pathogen of claim 31.

33. A method of inducing an immune response in an individual against an immunogen comprising administering to said individual:

said immunogen and/or a nucleic acid molecule comprising a nucleotide sequence that encodes said immunogen operably linked to regulatory elements; and

an immunomodulating protein and/or a nucleic acid molecule comprising a nucleotide sequence that encodes said immunomodulating protein operably linked to regulatory elements, wherein said immunomodulating protein is selected from the group consisting of: MCP-1, MIP-1α, MIP-1β, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, and Caspase ICE.

34. The method of claim 33 wherein said immunogen is a target protein that encodes a pathogen antigen, a cancer-associated antigen or an antigen linked to cells associated with autoimmune diseases.

35. The method of claim 33 wherein said immunogen is a pathogen antigen.

36. The method of claim 33 wherein said immunogen is an HIV-1 antigen.

37. The method of claim 33 wherein said immunomodulating protein is ICAM-1 and said method further comprises administering CD86 protein or a nucleotide sequence that encodes CD86 protein operably linked to regulatory elements.

38. An injectable pharmaceutical composition comprising a therapeutically effective amount of an antibody which specifically binds to an immunomodulating protein is selected from the group consisting of: MCP-1, MIP-1α, MIP-1β, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, and Caspase ICE.

39. A method of treating an individual who has an autoimmune disease comprising the step of administering to said individual an injectable pharmaceutical composition according to claim 38.

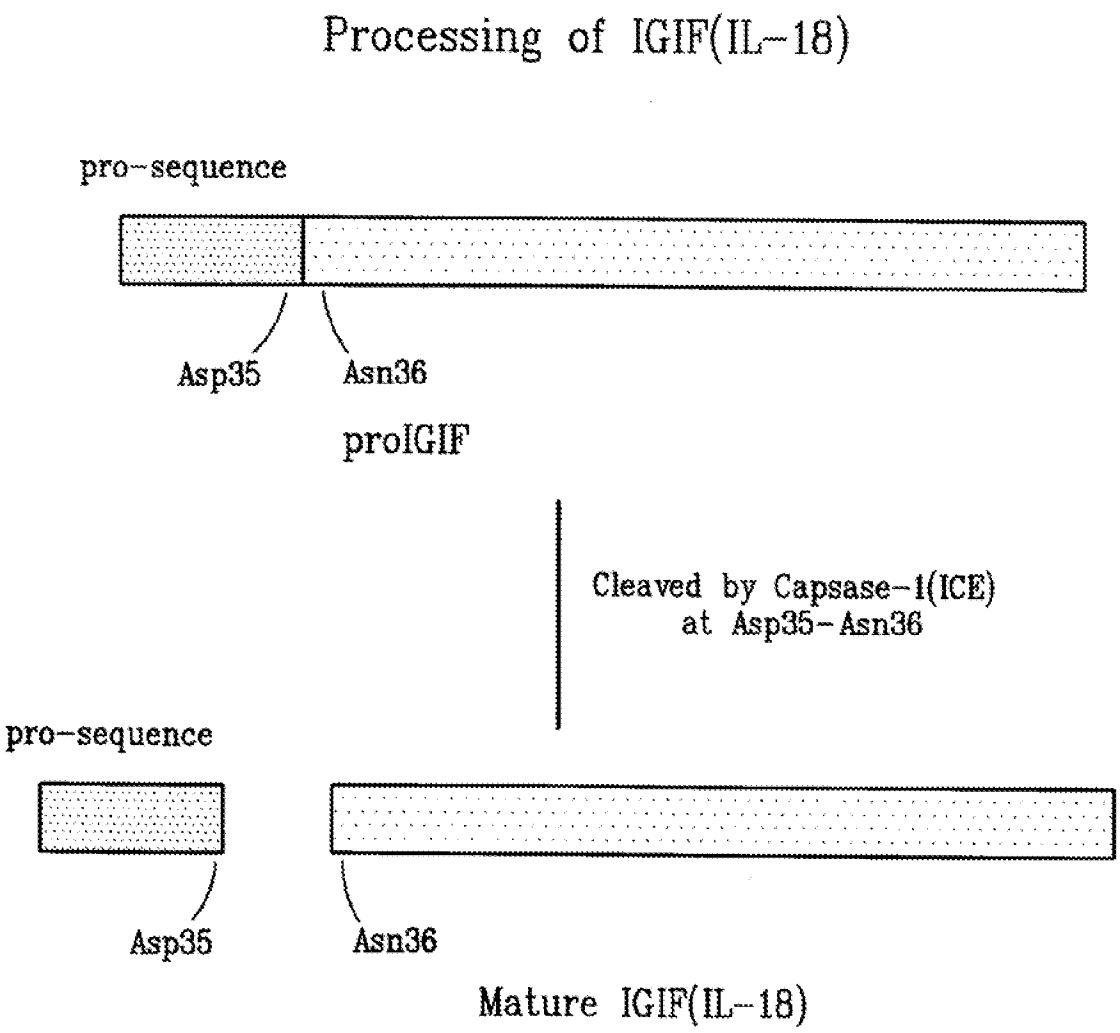
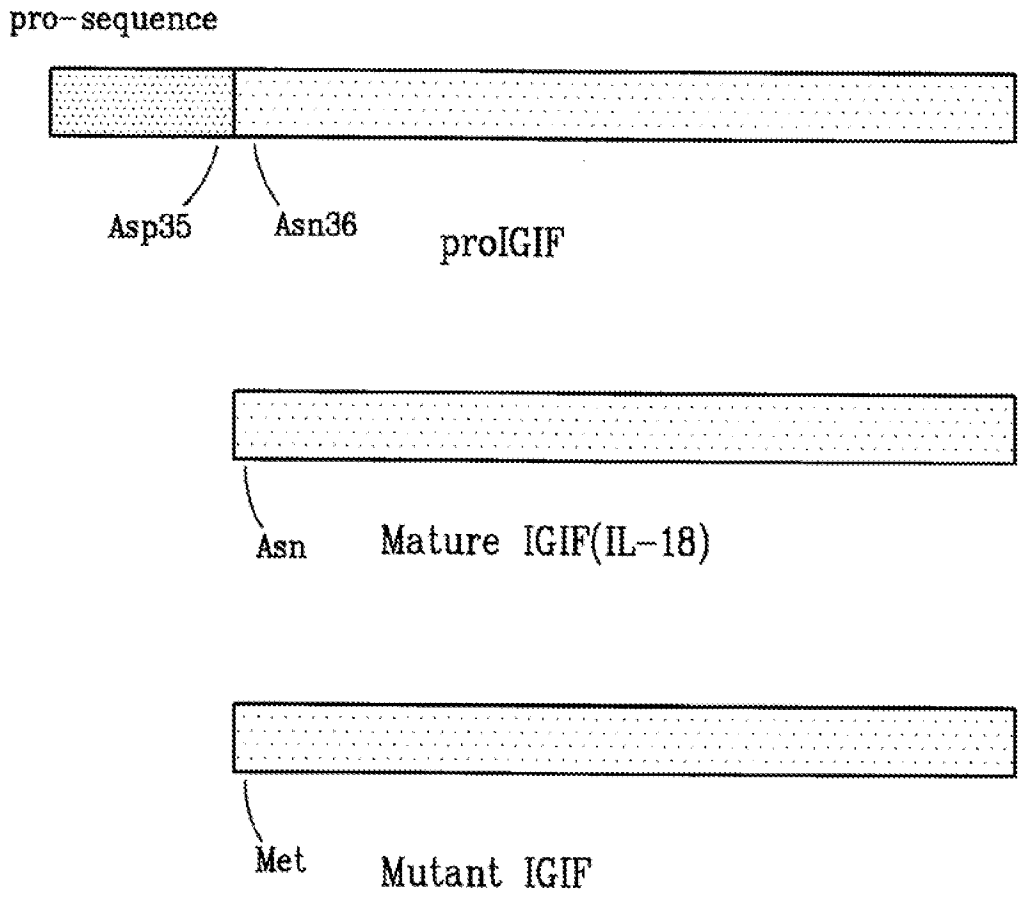

Processing of IGIF(IL-18)
pro-sequence
Asp35
Asn36
proIGIF
Cleaved by Capsase-1(ICE)
at Asp35-Asn36
pro-sequence
Asp35
Asn36
Mature IGIF(IL-18)

FIG. 1A



Mutant IGIF with the first amino acid mutated to Met can potentially bypass Capsase-1 processing hence be more potent

FIG. 1B

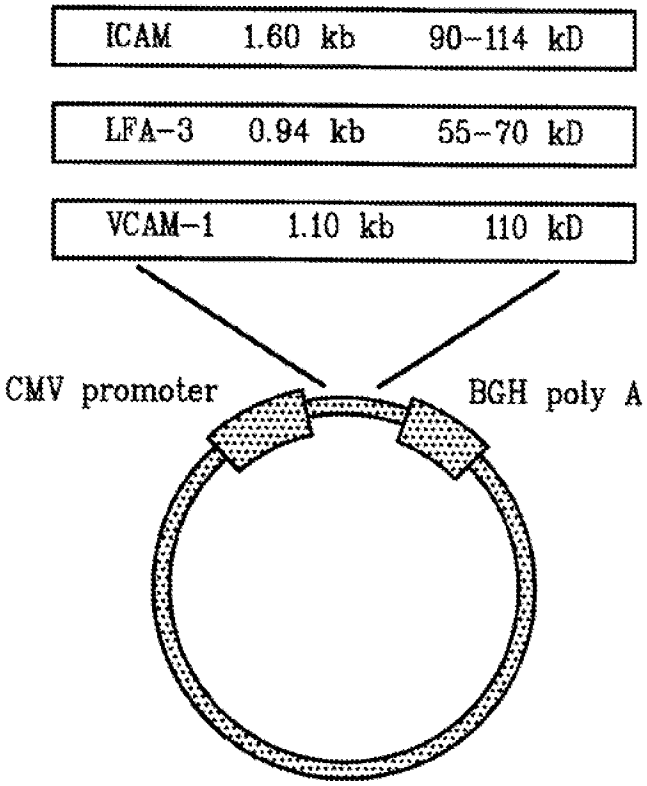

ICAM 1.60 kb 90-114 kD
LFA-3 0.94 kb 55-70 kD
VCAM-1 1.10 kb 110 kD
CMV promoter
BGH poly A

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/04332

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12N 15/85; A61K 48/00
US CL : 435/320.1; 514/44; 424/93.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/320.1; 514/44; 424/93.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RAMSHAW. I. et al. Expression of Cytokines by Recombinant Vaccinia Viruses: A Model for Studying Cytokines in Virus Infections in vivo. Immunol. Rev. 1992, Vol. 127, pages 157-178, especially page 159.	1-39
Y	XIANG. Z. et al. Manipulation of the immune response to a plasmid-encoded viral antigen by coinoculation with plasmids expressing cytokines. Immunity. February 1995, Vol. 2, pages 129-135, especially page 129.	1-39
X --- Y	CHOW. Y.-H. et al. Development of Th1 and Th2 populations and the nature of immune responses to hepatitis B virus DNA vaccines can be modulated by codelivery of various cytokine genes. J. Immunol. February 1998, pages 1320-1329, especially page 1320.	12-13, 17-18 --- 1-11, 14-16, 19-39

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 APRIL 1999	Date of mailing of the international search report 13 MAY 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ANNE MARIE S. BECKERLEG JOYCE BRIDGERS PARALEGAL SPECIALIST CHEMICAL MATRIX Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/04332

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TSUJI. T. et al. HIV-1-specific cell-mediated immunity is enhanced by co-inoculation of TCA3 expression plasmid with DNA vaccine. Immunology. 1997, Vol. 90, pages 1-6, especially page 1.	1-39

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/04332

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG: Medline, Biosis, Embase, Scisearch, Cancerlit, APS, Derwint WPX
searcg terms: plasmid, vector, vaccinia, retrovirus, adenovirus, cytokine, CD40 or MIP or rantes or ICAM, HIV or HSV or Influenza